August 2014

Important Note

Dear QIAsymphony PAXgene Blood RNA Kit user,

A temperature of –80°C is usually recommended for storing isolated RNA. Please note that we also recommend storing RNA purified with the QIAsymphony® PAXgene® Blood RNA Kit at a temperature of –80°C.

Please note that Buffer RDD, provided in the QIAsymphony PAXgene Blood RNA Kit, is equivalent to Buffer QSX1, as it is named in the QIAsymphony SP software. If you are using the Wizard or if Buffer RDD has not been scanned and correctly placed, the QIAsymphony SP will request that you place Buffer QSX1 on the instrument. In this case, place Buffer RDD from the QIAsymphony PAXgene Blood RNA Kit in the position specified by the QIAsymphony SP for Buffer QSX1.

Please also note that the DNase I solution should be placed in position 3 of the enzyme rack, labeled "ETDN" and not in position 2, which is used for the second proteinase K vial. The *QIAsymphony PAXgene Blood RNA Kit Handbook* (Second Edition, January 2012, page 16) incorrectly refers to position 2 for the DNase I solution. The enzyme rack is correctly labeled with "ETDN" for the DNase I solution at position 3.

For further information, please contact one of the QIAGEN Technical Service Departments or distributors (see the back cover of our handbooks or visit www.qiagen.com).

Best regards,

PreAnalytiX

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PreAnalytiX
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QIAsymphony® PAXgene®

Blood RNA Kit Handbook

For purification of cellular RNA from whole blood using the QIAsymphony SP instrument. To be used only in conjunction with PAXgene Blood RNA Tubes

When the QIAsymphony PAXgene Blood RNA Kit is used in conjunction with the PAXgene Blood RNA Tube, the System provides purified intracellular RNA, including miRNA for research tests including but not limited to RT-PCR.

The QIAsymphony PAXgene Blood RNA System is intended for Research Use Only. Not for use in diagnostic procedures.

Second Edition
January 2012

PreAnalytiX
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Trademarks:

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Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the QIAsymphony PAXgene Blood RNA Kit to the following terms:

1. The QIAsymphony PAXgene Blood RNA Kit may be used solely in accordance with the *QIAsymphony PAXgene Blood RNA Kit Handbook* and for use with components contained in the Kit only. PreAnalytiX grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the *QIAsymphony PAXgene Blood RNA Kit Handbook* and additional protocols available at www.preanalytix.com.
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PreAnalytiX Company

PreAnalytiX GmbH
Feldbachstrasse
CH – 8634 Hombrechtikon
Switzerland
www.preanalytix.com

PreAnalytiX Distributors

PreAnalytiX products are manufactured for PreAnalytiX by QIAGEN or BD and are distributed for PreAnalytiX by QIAGEN or BD. Products cannot be ordered at PreAnalytiX GmbH.

Please see the last pages for contact information for your local PreAnalytiX distributor.

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Intended Use

For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. The performance characteristics of this product have not been fully established.

All due care and attention should be exercised in the handling of the products.

The QIAsymphony PAXgene Blood RNA Kit is intended for purification of intracellular RNA, including miRNA, from human whole blood (4.8×10^6 – 1.1×10^7 leukocytes/ml).

The QIAsymphony PAXgene Blood RNA Kit is not for the isolation of genomic DNA or viral nucleic acids from human whole blood.

Summary and Explanation

QIAsymphony technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples.

The QIAsymphony PAXgene Blood RNA Kit allows automated, standardized purification on the QIAsymphony SP of total RNA, including miRNA, from 2.5 ml human whole blood collected into PAXgene Blood RNA Tubes. Proven MagAttract® magnetic-particle technology provides high-quality RNA, which is suitable for direct use in downstream applications. The QIAsymphony SP performs all steps of the sample preparation procedure. Up to 72 samples, in 3 batches of 24, can be processed in a single run.

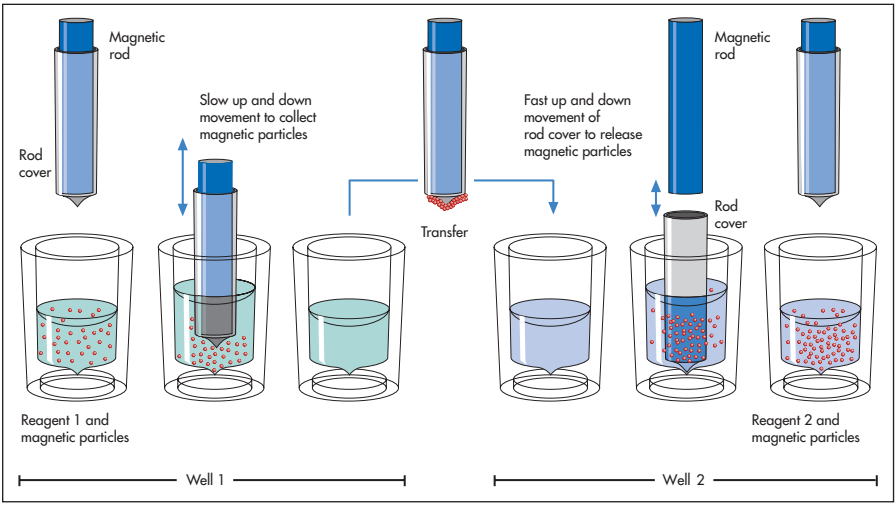
Magnetic rod
Rod cover
Slow up and down movement to collect magnetic particles
Transfer
Fast up and down movement of rod cover to release magnetic particles
Magnetic rod
Rod cover
Reagent 1 and magnetic particles
Reagent 2 and magnetic particles
Well 1
Well 2

Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

Principle of the Procedure

The simple procedure begins with a centrifugation step to pellet the contents of each PAXgene Blood RNA Tube. After decanting the supernatant, the pellets are then resuspended by vortexing in optimized buffers, which are supplemented with proteinase K. The tubes are transferred to the QIAsymphony SP.

After a precleaning step, RNA binds to the silica surface of MagAttract magnetic particles in the presence of a chaotropic salt and isopropanol. Two prewash steps guarantee that DNase I digestion is optimized. Remaining proteins and contaminants are removed by an additional proteinase K treatment and 3 wash steps, and RNA is eluted in Buffer BR5. Finally, a heat treatment of the eluted RNA provides ready-to-use RNA and ensures consistent performance in downstream applications.

Guaranteed yields of RNA isolated from 2.5 ml healthy, human, whole blood (4.8 x 10^6 – 1.1 x 10^7 leukocytes/ml) are ≥3 µg for >95% of the samples processed. Since yields are highly donor-dependent, individual yields may vary. Average yields can be expected in the range between 8 and 16 µg. Purified RNA can be used in downstream applications including but not limited to RT-PCR.

Materials Provided

Kit contents

QIAsymphony PAXgene Blood RNA Kit	**(96)**
Catalog no.	**762635**
Number of preps	**96**
Buffer BR2	25 ml
Buffer BR5	30 ml
Buffer QSX2	30 ml
Buffer RDD	30 ml
Proteinase K	2 x 1.4 ml
DNase I (1500 Kunitz units)*	4 glass vials
RNase-Free Water	2 x 2 ml
Reagent Cartridge†	2
Piercing Lid	2
Reuse Seal Set	2
Secondary Hemogard Closures	2 x 50
Elution Microtubes CL, racked‡	2
Caps for Elution Microtubes‡	55 x 8
Handbook	1

* Kunitz units are the commonly used units for measuring DNase I; see page 16 for definition.

† Prefilled reagent cartridges include buffers that contain a guanidine salt, ethanol, isopropanol, and/or proteinase K. See pages 8–10 for safety information.

‡ Also available separately. See page 29 for ordering information.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Consumables

- 8-Rod Covers (cat. no. 997004)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Sterile, aerosol-barrier, RNase-free pipet tips*
- RNase-free syringe and needle (for resuspension of DNase I)
- Crushed ice

Reagents

- PAXgene Blood RNA Tubes (cat. no. 762165; available from BD and BD authorized distributors; see www.PreAnalytiX.com)

Equipment

- QIAsymphony SP (cat. no. 9001297)
- Optional: QIAsymphony Cabinet SP (cat. no. 9020244)
- Pipets† (200 µl – 1 ml)
- Centrifuge† capable of attaining 3000–5000 x *g* and equipped with a swing-out rotor and buckets to hold PAXgene Blood RNA Tubes
- Vortex mixer†
- Optional: A step dispenser,† such as the Multipette plus from Eppendorf, is recommended for optimal processing‡
- Optional: A multitube vortexer,† such as the VX2500 from VWR, is recommended for optimal processing‡

* Ensure that you are familiar with the guidelines on handling RNA (Appendix A, page 25).

† Ensure that instruments have been checked and calibrated regularly according to the manufacturer's recommendations.

‡ This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Elution in Elution Microtubes CL

Equipment

- PAXgene 96 Incubator Block (cat. no. 9238279)
- Incubator capable of 80°C*
- Spatula to remove the lower plate from the elution microtube rack
- Heavy plate to prevent caps from opening during incubation at 80°C

Elution in 2 ml Sarstedt tubes

Consumables

- Micro tube 2ml, PP (Sarstedt, cat. no. 72.608) with Screw cap, neutral (Sarstedt, cat. no. 65.716.725)†

Equipment

- Shaker–incubator, heating block, or water bath* capable of incubating at 65°C

For other possible elution formats see
www.qiagen.com/products/qiasymphonysp.aspx under user support.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.preanalytix.com/RNA_msds.asp where you can find, view, and print the MSDS for this kit.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The bottles and the troughs of the reagent cartridges contain Buffer BR2 and Buffer QSB1 which contain guanidine thiocyanate, and Buffer QSW5, which contains guanidine hydrochloride. Guanidine thiocyanate and guanidine hydrochloride can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

* Ensure that instruments have been checked and calibrated regularly according to the manufacturer's recommendations.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Sample-preparation waste, such as supernatants from centrifugation steps in the RNA purification procedure, is to be considered potentially infectious. Dispose of potentially infectious waste according to your institution's waste-disposal guidelines.

The following risk and safety phrases apply to components of the QIAsymphony PAXgene Blood RNA Kit.

Buffer BR2 (bottles and trough 1 of the reagent cartridges)

Xn

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer QSB3 (trough 2 and 3 of the reagent cartridges)

F, Xi

Contains isopropanol: highly flammable, irritant. Risk and safety phrases:* R11-36-67, S13-26-36/37/39-46

Buffer QSB1 (troughs 4 of the reagent cartridges)

F, Xn

Contains isopropanol and guanidine thiocyanate: highly flammable, harmful, irritant. Risk and safety phrases:* R11-20/21/22-32-36-67, S13-26-36/37/39-46

Buffer QSW5 (trough 5 of the reagent cartridges)

F, Xn

Contains ethanol and guanidine hydrochloride: highly flammable, harmful, irritant. Risk and safety phrases:* R11-22-36/38, S13-26-36/37/39-46

* R11: Highly flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36: Irritating to eyes; R36/38: Irritating to eyes and skin; R67: Vapours may cause drowsiness and dizziness; S13: Keep away from food, drink and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Buffer BR4 (trough 6 of the reagent cartridges)

F

Contains ethanol: highly flammable. Risk phrase:* R11 S7-16

Proteinase K (in tubes and in the enzyme racks of the reagent cartridges)

Xn

Contains proteinase K *(Tritirachium album)*: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23 24 26 36/37

DNase I

Xn

Contains deoxyribonuclease (bovine): sensitizer. Risk and safety phrases:* R42/43, S22-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Reagent Storage and Handling

Except for the DNase I vials, the remaining components of the QIAsymphony PAXgene Blood RNA Kit can be stored at room temperature (15–25°C). Do not store reagent cartridges at temperatures below 15°C.

The QIAsymphony PAXgene Blood RNA Kit contains a ready-to-use proteinase K solution in the enzyme rack of the reagent cartridge (see Figure 2, page 12) and as separate tubes. Proteinase K can be stored at room temperature (15–25°C). To store for extended periods of time, we suggest keeping the enzyme rack with proteinase K and DNase I tubes at 2–8°C.

* R11: Highly flammable; R36/37/38: Irritating to eyes, respiratory system and skin; R42/43: May cause sensitization by inhalation and skin contact; S7: Keep container tightly closed; S16: Keep away from sources of ignition — No smoking; S23: Do not breathe fumes; S24: Avoid contact with the skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves.

The DNase I vials are shipped at room temperature. The vials should be stored immediately upon receipt at 2–8°C. When stored at 2–8°C and handled correctly, the lyophilized enzyme can be kept for at least 9 months without showing any reduction in performance. The DNase I stock solution in the enzyme rack has to be stored at 2–8°C (see "Things to do before starting", page 16, for preparation of DNase I stock solution).

For other possible elution formats see
www.qiagen.com/products/qiasymphonysp.aspx under user support.

Partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, seal the piercing lid with the Reuse Seal Set provided. To avoid evaporation, seal the reagent cartridge immediately after the end of the protocol run. The reagent cartridge is designed to allow 4 uses with a total opening time of up to 15 hours at a maximum environmental temperature of 30°C.

Specimen Handling and Storage

Blood must be collected in PAXgene Blood RNA Tubes (cat. no. 762165). See the *PAXgene Blood RNA Tube Product Circular* for information about specimen collection and handling. A video where all details of the correct handling of the PAXgene Blood RNA Tubes are shown is available on the PreAnalytiX Web site (www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/ under Resources — Videos).

After collection of the blood sample, it is important to incubate the PAXgene Blood RNA Tube for at least 2 hours at room temperature (15–25°C) before RNA purification. Incubation of the PAXgene Blood RNA Tube overnight may increase RNA yields in some cases. If the blood samples in the PAXgene Blood RNA Tubes were frozen, they must be thawed at room temperature for at least 2 hours before RNA purification.

Procedure

Automated purification on the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer. Start the protocol and remove purified RNA from the eluate drawer after sample processing is completed. Refer to the *QIAsymphony SP User Manual* for operating instructions.

Even though the QIAsymphony SP was designed as a benchtop instrument, the use of the QIAsymphony Cabinet SP will enhance the convenience of using this robotic system. The QIAsymphony Cabinet SP is specifically designed for correct positioning of the QIAsymphony SP instrument. The QIAsymphony Cabinet SP contains a waste compartment, into which used tips from the worktable can be ejected.

"Reagents and Consumables" drawer

Reagent cartridges

Reagents for purification of RNA from PAXgene Blood RNA Tubes are contained in an innovative reagent cartridge (Figure 2). Only buffer RDD has to be loaded in a separate bottle holder position. Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, enzyme buffer, binding buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later use. This avoids generating waste due to leftover reagents at the end of the purification procedure.

Figure 2. The reagent cartridge contains all reagents required for the protocol run.

Note: Before using a reagent cartridge for the first time, remove the seal from the trough containing the magnetic particles, and replace it with the trough cover. Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 minutes before first use. Place the reagent cartridge into the reagent cartridge holder. If required by the protocol, place the enzyme rack into the reagent cartridge holder. Place the piercing lid on top of the reagent cartridge (Figure 3). Scan bar code of the buffer RDD bottle and load it in the buffer bottle position.

Important: The piercing lid is sharp; use caution when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

The reagent cartridge is then loaded into the "Reagents and Consumables" drawer.

Figure 3. Easy worktable setup with reagent cartridges.

Loading plasticware

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips in blue racks, 1500 µl tips in gray racks) are loaded into the "Reagents and Consumables" drawer (see Table 1, page 15).

See Table 1 (page 15) for the consumables required. For plasticware ordering information, see page 29.

Note: Both types of tips have filters to prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will automatically identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

"Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer. If a QIAsymphony Cabinet SP is used, the waste compartment, into which used tips from the worktable are ejected, must be checked for sufficient space to collect waste tips.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the *QIAsymphony SP User Manual*.

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Furthermore, a liquid-level sensor detects the level of liquid in the waste container. The system notifies the user if there is not enough capacity left in the container for liquid waste from the queued batch.

"Eluate" drawer

The required elution rack is loaded into the "Eluate" drawer. Do not load a 96 well plate into "Elution slot 4". Use "Elution slot 1" with the corresponding cooling adaptor so that eluates will be cooled at the end of the run.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Table 1).

Table 1. Consumables required for purification of cellular RNA from whole blood

Number of samples	24	48	72
Reagent cartridges	1	1	2
Sample prep cartridges*	27	54	81
8-Rod Covers†	3	6	9
1500 µl tips‡ (tip racks)	132 (5)	264 (9)	396 (13)
200 µl tips‡ (tip racks)	28 (1)	56 (2)	84 (3)

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack; depending on the reagent cartridge status, the inventory scan requires additional tips (two 200 µl and nine 1500 µl tips).

Protocol: Purification of total RNA, including miRNA, from whole blood collected in PAXgene Blood RNA Tubes

Important points before starting

- Blood must be collected in PAXgene Blood RNA Tubes (cat. no. 762165).
- Use of a step dispenser, such as the Multipette plus from Eppendorf, and a multitube vortexer, such as the VX2500 from VWR, is recommended.
- All steps of the QIAsymphony PAXgene Blood RNA protocol for purification of total RNA, including miRNA, should be performed at 18–25°C.

Things to do before starting

- After collection of the blood sample, it is important to incubate the PAXgene Blood RNA Tube for at least 2 hours at room temperature (15–25°C) before RNA purification. Incubation of the PAXgene Blood RNA Tube overnight may increase RNA yields in some cases. If the blood samples in the PAXgene Blood RNA Tubes were frozen, they must be thawed at room temperature for at least 2 hours before RNA purification.
- Prepare DNase I stock solution before using the reagent cartridge for the first time. Dissolve 2 vials of the lyophilized DNase I (1500 Kunitz units* each) in 900 µl per vial of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Instead, inject RNase-free water into each vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex**. Transfer the contents of both vials (1.8 ml total) to the tube at position 2 of the enzyme rack on the reagent cartridge.

  DNase solution can be stored at 2–8°C for up to 6 weeks.
- Buffer BR2 (reagent trough 1 and separate bottle) may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- If using a new reagent cartridge, remove the seal from the trough containing the magnetic particles. Make sure that the piercing lid is placed on the reagent cartridge.
- If using a previously used reagent cartridge, make sure that the Reuse Seal Strips and trough cover have been removed.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. **33**, 349 and 363).

- The enzyme rack must be attached to the reagent cartridge, and the tube caps must be removed.
- Orient sample racks so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- We recommend using Elution Microtubes CL, racked (provided) for elution. Alternatively, 2 ml Sarstedt tubes can be used (see page 8). For other possible elution formats see www.qiagen.com/products/qiasymphonysp.aspx under user support.

Procedure

1. **Centrifuge the PAXgene Blood RNA Tubes for 10 minutes at 3000–5000 x *g* using a swing-out rotor.**

   Note: Use only round-bottomed tube adapters. Tubes may break during centrifugation if centrifuge adaptors with conical bottoms are used.

   To save time, the QIAsymphony SP can be set up (steps 7 to 10) during this centrifugation step.

2. **After centrifugation, remove the supernatant by decanting. Discard the supernatant, and save the pellet for resuspension in step 3.**
3. **Mix 280 µl buffer QSX2 with 20 µl proteinase K per sample.**

   Note: For 24 (48, 72) samples, mix 7 (14, 21) ml of buffer QSX2 with 500 (1000, 1500) µl proteinase K.

4. **Add 300 µl Buffer QSX2–proteinase K mixture per tube. Close the tubes with the Secondary Hemogard Closures provided, and thoroughly resuspend the pellet by vortexing. For resuspension use a multitube vortexer at full speed for 30 seconds or until the pellets are completely resuspended.**
5. **Remove and discard the closures.**
6. **Add 200 µl Buffer BR2 per tube and place them into the appropriate sample carrier.**
7. **Ensure that the QIAsymphony SP is switched on.**

   The power switch is located at the bottom left corner of the QIAsymphony SP.

8. **Ensure that the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
9. **Load the required reagent cartridge and consumables (see Table 1, page 15) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**

   You must scan the bar code of the Buffer RDD bottle before placing it into the "Reagents and Consumables" drawer. To do this, choose the "Bottle ID" button in the "Consumables" dialog box on the touchscreen.

10. **Load the required elution rack into the "Eluate" drawer.**

    Place the adapter required for the specific elution format onto the selected elution position. If Elution Microtubes CL are used in the eluate cooling position ("Elution Slot 1"), remove the lower plate from the elution microtube rack using a spatula. Do not load a 96-well plate onto "Elution slot 4".

11. **Load the samples (from step 6) into the "Sample" drawer.**

12. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

    Enter the following information:

    - Sample information (change default tube format; choose the "Select all" button on the sample view screen, and select "BD # 762165 PAXgene RNA 16x100" from the "Tube Insert 00" sheet)
    - Protocol ("Assay Control Set") to be run
    - Elution volume and output position

    After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

13. **Press the "Run" button to start processing.**

    All processing steps are fully automated.

    The time elapsed is displayed.

    At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

    It is recommended to use the "Elution slot 1" because this slot is able to cool the eluates after the run is complete.

14. **After the QIAsymphony protocol finishes, remove the elution microtubes or 2 ml Sarstedt tubes containing the purified RNA, and seal them with the appropriate caps.**

    If the "Eluate" drawer is opened and not reclosed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message window appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

    Result files are generated for each elution plate.

15. **Follow step 15a if the RNA was eluted into Elution Microtubes CL or step 15b if the RNA was eluted into 2 ml Sarstedt tubes.**

    If using Elution Microtubes CL, heat an incubator to 80°C for use in step 15a. If using 2 ml Sarstedt tubes, heat a shaker–incubator, heating block, or water bath to 65°C for use in step 15b.

15a. Place the elution microtube rack onto the PAXgene 96 Incubator Block preheated in the 80°C incubator, and incubate for 10 minutes at 80°C. Place a heavy plate over the caps to prevent them from popping open. After incubation, proceed immediately with step 16.

Note: Denaturation of the eluate is essential for maximum efficiency in downstream applications, such as RT-PCR, other amplification reactions, or cDNA synthesis. It is not necessary to denature samples more than once; samples remain denatured after freezing and thawing. Do not exceed the incubation time or temperature.

15b. Incubate the eluates for 5 minutes at 65°C in a shaker–incubator, heating block, or water bath without shaking. After incubation, proceed immediately with step 16.

Note: Denaturation of the eluate is essential for maximum efficiency in downstream applications, such as RT-PCR, other amplification reactions, or cDNA synthesis. It is not necessary to denature samples more than once; samples remain denatured after freezing and thawing. Do not exceed the incubation time or temperature.

If elution positions 2 or 3 were used (position 4 is not recommended for 96-well elution formats), you must remove the bottom plate of the elution microtube rack with a spatula first.

16. After incubation, chill the elution microtubes or Sarstedt tubes immediately on ice. Put the bottom plate back onto the rack for storage. Store the purified RNA at –15 to –30°C or at –80°C.

For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting the sample in 10 mM Tris·Cl, pH 7.5 buffer. Dilution of the sample in RNase-free water may lead to inaccurately low values. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer BR5 as the volume of eluted RNA to be diluted.

Note: For quantification in Tris buffer, use the relationship

$A_{260} = 1 \Rightarrow 44$ µg/ml.

In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should be spun down, and eluates should be transferred to a clean tube. Alternatively, a suitable magnet can be used (see Appendix B, page 25).

17. If the reagent cartridge is only partially used, seal it with the Reuse Seal Strips (provided), and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation. Remove the enzyme rack and store it at 2–8°C.

18. **Discard used sample tubes and waste according to your local safety regulations.**
    See pages 8–10 for safety information.
19. **Clean the QIAsymphony SP.**
    Follow the maintenance instructions in the *QIAsymphony SP User Manual*.
20. **Close the workstation drawers, and switch off the QIAsymphony SP.**

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at www.preanalytix.com/product-catalog/blood/rna/products/qiasymphony-paxgene-blood-rna-kit/ under Resources — FAQs.

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
General handling		
	Error message displayed in the touchscreen	IIf an error message is displayed during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP User Manual.*
RNA degraded		
a)	RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 25). Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
b)	Incorrect blood donation	Strictly follow the instructions in the P*AXgene Blood RNA Tubes Product Circular*. A video where all details of the correct handling of the PAXgene Blood RNA Tubes are shown is available on the PreAnalytiX Web site (www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/ under Resources — Videos).
RNA does not perform well in downstream applications		
a)	Salt carryover during elution	Ensure that the reagent cartridge is at room temperature (15–25°C).

		Comments and suggestions
b)	Eluate concentrated by vacuum centrifugation	Do not concentrate the eluate by vacuum centrifugation (e.g., in a SpeedVac® or similar instrument). This can introduce RNases, lead to degradation due to high temperatures, and concentrate salts in the eluate, which can interfere with downstream applications.
c)	Insufficient RNA used in downstream application	Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see Appendix B, page 25).
d)	Excess RNA used in downstream application	Excess RNA can inhibit some enzymatic reactions. Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see Appendix B, page 25).
e)	Bead carryover	In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If very high portions of eluates are needed for specific downstream assays, eluates could be spun down, and eluates should be transferred to a clean tube.
Low RNA yield		
a)	Less than 2.5 ml blood collected in the PAXgene Blood RNA Tube	Ensure that 2.5 ml blood is collected in the PAXgene Blood RNA Tube (see the *PAXgene Blood RNA Tube Product Circular*). A video where all details of the correct handling of the PAXgene Blood RNA Tubes are shown is available on the PreAnalytiX Web site (www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/ under Resources — Videos).
b)	RNA concentration measured in water	RNA concentration should be measured in 10 mM Tris·Cl, pH 7.5, for accurate quantification (see Appendix B, page 25).

		Comments and suggestions
c)	Pellet overdried in step 2	After removing the supernatant by decanting, it is sufficient to dab the rim of the tube 5–10 times with a clean paper towel. Excess drying (such as placing the tubes upside down in a rack or wiping the inner walls of the tube) is not recommended.
d)	Blood incubated for <2 hours after collection	Incubate blood in the PAXgene Blood RNA Tube for at least 2 hours after collection. Incubation of the PAXgene Blood RNA Tube overnight may increase yields slightly in some cases.
e)	Low white blood cell count	RNA yields are highly donor-dependent. Blood samples with low leukocyte counts (e.g., <4.8 x 10^6 leukocytes/ml) will give low yields.
f)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
Low A_{260}/A_{280} ratio		
a)	Wrong buffer used for RNA dilution	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute RNA before measuring purity (see "Purity of RNA", page 27).
b)	Spectrophotometer not properly zeroed	To zero the spectrophotometer, use a blank containing the same proportion of elution buffer (Buffer BR5, provided in an extra bottle with the kit) and dilution buffers as in the samples to be measured. Components in buffers may affect the A_{260}/A_{280} ratio.
c)	Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see "Quantification of RNA", page 25).
Precipitate in reagent trough of opened cartridge		
	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for RNA purification.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony PAXgene Blood RNA Kit is tested against predetermined specifications to ensure consistent product quality.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN and PreAnalytiX products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Contact Information

For technical assistance and more information, call one of the QIAGEN Technical Service Departments or local distributors (see last page or visit www.qiagen.com).

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Protocols for removing RNase-contamination from glassware and solutions can be found in general molecular biology guides, such as Sambrook, J. and Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Appendix B: Quantification and Determination of Quality of Total RNA

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of total RNA", below). Small amounts of RNA can be quantified using the Agilent® 2100 Bioanalyzer, fluorometric quantification, or quantitative, real-time RT-PCR.

Spectrophotometric quantification of total RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per milliliter (A_{260} = 1 ⇒ 44 µg/ml). For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting the sample in 10 mM Tris·Cl, pH 7.5. Dilution of the sample in RNase-free water may lead to inaccurately low values. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer BR5 as the volume of eluted RNA to be diluted. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. Extra buffer BR5 to zero the spectrophotometer is provided in a separate bottle with the QIAsymphony PAXgene Blood RNA Kit.

When measuring RNA samples, be certain that cuvettes or plates are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes or plates with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water.

Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the RNA in downstream applications. If it is necessary to minimize magnetic-particle carryover, the tube containing the eluate should first be placed in a suitable magnet (e.g., QIAGEN® 12-Tube Magnet, cat. no. 36912, for 1 minute) and the eluate transferred to a clean tube. If a suitable magnet is not available, centrifuge the tube containing the RNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.

When quantifying RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 80 µl

Dilution = 10 µl of RNA sample + 140 µl 10 mM Tris·Cl, pH 7.5 (1/15 dilution)

Measure absorbance of diluted sample in a cuvette (RNase-free).

A_{260} = 0.3

Concentration of RNA sample	= 44 x A_{260} x dilution factor
	= 44 x 0.3 x 15
	= 198 µg/ml
Total yield	= concentration x volume of sample in milliliters
	= 198 µg/ml x 0.08 ml
	= 15.8 µg RNA

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Quantification of miRNA

Since the RNA eluate obtained using this procedure contains various small RNA species, the yield of specific small RNA species (e.g., miRNA) cannot be quantified by OD measurement or fluorogenic assays. Instead, we recommend using quantitative, real-time RT-PCR assays, such as the miScript PCR System, specific for the type of small RNA under study. For example, to estimate miRNA yield, an assay directed against any miRNA known to be adequately expressed in the samples being processed may be used.

The miScript PCR System is a three-component system that covers all the steps of conversion of miRNA and mRNA into cDNA and detection of miRNAs in SYBR® Green–based real-time PCR. A single cDNA synthesis reaction is sufficient for analysis of multiple miRNAs. The miScript PCR System can also be used for detection of other small RNAs, such as snoRNAs or piRNAs. See page 29 for ordering information.

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.8–2.2† in 10 mM Tris·Cl, pH 7.5. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer BR5 as the volume of eluted RNA to be diluted. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

When determining the purity of RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Purity of RNA sample = $(A_{260} - A_{320}) / (A_{280} - A_{320})$

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. To prevent any interference by DNA in RT-PCR applications, we recommend working with intron-spanning primers so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels following RT-PCR by performing control experiments in which no reverse transcriptase is added before the PCR step.

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† Values up to 2.4 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

Integrity of RNA

The integrity and size distribution of total RNA purified with the QIAsymphony PAXgene Blood RNA Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide* staining or by using capillary electrophoresis systems like the Agilent 2100 Bioanalyzer. The corresponding ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S RNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation either before or during preparation.

The Agilent 2100 bioAnalyzer also provides an RNA Integrity Number (RIN) as a measure of RNA integrity. Especially for samples that contain large amounts of small RNAs the RIN is not the method of choice to check RNA integrity because these small RNAs are calculated as degraded fragments.[†]

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† Voss, T., Kruhøffer, M., Nielsen, K.B., Heese, F., and Wyrich, R. (2010) Automated, magnetic bead miRNA enrichment from stabilized whole blood: Development of an optimized protocol and comparison to established procedures by the SPIDIA consortium. Association for Molecular Pathology Annual Meeting, San Jose, USA. See references.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony PAXgene Blood RNA Kit (96)	For 96 preps: 2 Reagent Cartridges, Enzyme Racks, Accessories, and RNase-Free Buffers	762635
QIAsymphony SP	QIAsymphony sample prep module, 1 year warranty on parts and labor	9001297
Accessories		
PAXgene 96 Incubator Block	Block for denaturation of eluates in PAXgene procedures	9238279
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw-cap tubes; for use with the QIAsymphony SP	9018577
Cooling Adapter, 2ml, v2, Qsym	Cooling adapter for 2 ml screw-cap tubes; for use with the QIAsymphony SP/AS instruments (software version 3.1)	9020674
Sample Prep Cartridges, 8-well (336)	8-well Sample Prep Cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 1500 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	997024
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990332
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes; 2304 in racks of 96; includes cap strips	19588
QIAsymphony AS	QIAsymphony assay setup module, 1 year warranty on parts and labor	9001301
QIAsymphony Cabinet SP	Accessory for correct positioning of the QIAsymphony SP instruments	9020244

Ordering Information

Product	Contents	Cat. no.
12-Tube Magnet	Magnet for separating magnetic beads in 12 x 1.5 ml or 2 ml tubes	36912
Related products		
QIAsymphony RNA Kit — for purification of total RNA from animal and human cells and tissues using the QIAsymphony SP		
QIAsymphony RNA Kit	For 192 preps: 2 Reagent Cartridges, Enzyme Racks, and Accessories	931636
miScript PCR System — for quantification of mature miRNA, precursor miRNA, and mRNA from the same cDNA sample		
miScript Reverse Transcription Kit (50)	For 50 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218061
miScript SYBR Green PCR Kit (1000)	For 1000 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218075
Human miScript Assay 96 Set V13.0 (20)	846 miScript Primer Assays (for 20 x 50 µl reactions) targeting human miRNAs in miRBase version 13.0; provided in 96-well plates; 6 miScript PCR Controls on each plate	218421
Rotor-Gene® Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM® System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX or QIAGEN kit handbook or user manual. PreAnalytiX and QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

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